

Appl. No. 10/694,641
Amdt. dated June 14, 2005
Preliminary Amendment

PATENT

Amendments to the Specification:

On page 17, please add the following new paragraph [0015A] immediately after line 5:

[0015A] Figure 6 shows the metabolic pathway for linoleic acid, the most abundant fatty acid in the average American diet. As shown in the Figure, linoleic acid can be epoxidized at either or both of the double bonds. The epoxides can be converted by the soluble epoxide hydrolase into 1,2-diols which can then be conjugated.

Please replace paragraph [0062] with the following amended paragraph:

[0062] Animals. Male SHR and WKY rats 3-13 wks of age were purchased from Charles River Laboratories (Wilmington, MA) and housed in a controlled environment with 12 hr light/dark cycles and fed standard laboratory chow for ~~3~~ 3 days before euthanasia. All animal use was approved by the University of California San Francisco Committee on Animal Research and followed the National Institutes of Health ~~guidelines~~ guidelines for the care and use of laboratory animals. For isolation of kidney subcellular fractions, rats were anesthetized with methoxyflurane, the abdominal cavities were opened, and the kidneys were perfused with ice-cold saline. Perfused kidneys were rapidly removed, the cortex and medulla dissected out and immersed in liquid nitrogen. All tissue was stored at -80 °C until preparation of microsomes. In some cases WKY and SHR rats were housed in metabolic cages for up to three days and urine was collected over triphenylphosphine in 24 hr intervals. The urine volume was noted and aliquots were stored at -80 °C prior to extraction and quantitation of DHETs and EETs. For the sEH inhibition studies, groups of 8 wk old male SHRs and WKY rats were treated daily for 1-4 days with a 3 mg/kg i.p. dose of N,N'-dicyclohexylurea (DCU) in a 1.5:1 mixture of corn oil and DMSO. Systolic blood pressure was measured at room temperature by a photoelectric tail cuff system (Model 179, IITC, Inc., Woodland Hills, CA) for up to four days following the dose of inhibitor. Blood pressures are reported as the average of three separate readings over a 30 min period. Urine was collected for 24 hr immediately following a dose of DCU or vehicle for quantification of DHET and EET excretion. Similar inhibition studies were carried out with

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equimolar doses of N-cyclohexyl-N'-dodecylurea, N-cyclohexyl-N'-ethylurea and dodecylamine.

Please replace paragraph [0065] with the following amended paragraph:

[0065] EET hydrolysis. Racemic [1-14C]EETs were synthesized and purified according to published methods from [1-14C]arachidonic acid (56-57 $\mu\text{Ci}/\mu\text{mole}$) by nonselective epoxidation (Falck, J.R. et al., *Meth Enzymol* 187, 357-364 (1990)). Hydrolysis of [1-14C]EETs was measured in WKY and SHR renal S9 fractions at 37°C as described previously (Zeldin, D.C. et al. *J Biol Chem* 268, 6402-6407 (1993)). The reaction mixture consisted of 50 μM EET (0.045-0.09 μCi) and 1 mg/ml S9 protein (0.5 mg/ml SHR S9 protein for 14,15-EET hydrolysis) in 150 mM KCl, 10 mM MgCl_2 , 50 mM potassium phosphate buffer pH 7.4. Reactions were carried out for 40 min (10 min for 14,15-EET hydrolysis in SHR samples) and the reaction products were extracted into ethyl acetate, evaporated under a blanket of nitrogen and detected by reverse phase HPLC with radiometric detection as described for the arachidonic acid incubations.

Please replace paragraph [0074] with the following amended paragraph:

[0074] A tight binding sEH specific inhibitor, dicyclohexylurea (DCU) (Morisseau, C. et al., *Proc Natl Acad Sci USA* 96, 8849-8854 (1999)), was used to reduce sEH activity in vivo and to determine the effect of decreased EET hydrolysis on blood pressure. Inhibition of EET hydrolysis by DCU was confirmed in incubations of renal S9 fractions with the regioisomeric EETs (Figure 4A). A dose-dependent inhibition of EET hydrolysis by DCU was apparent for all three regioisomers. DCU had the most significant effect on the hydrolysis of 8,9-EET, inhibiting this reaction with an IC_{50} of $0.086 \pm 0.014 \mu\text{M}$. The corresponding IC_{50} values for inhibition of 11,12- and 14,15-EET hydrolysis were $0.54 \pm 0.08 \mu\text{M}$ and $0.45 \pm 0.16 \mu\text{M}$, respectively. At concentrations up to 25 μM , DCU had no effect on CYP epoxygenase or ω -hydroxylase activity and previous studies from our laboratory have shown that DCU does not inhibit mEH (Morisseau, C. et al., *Proc Natl Acad Sci USA* 96, 8849-8854 (1999)). The potent

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inhibition of sEH by DCU was confirmed with purified recombinant rat sEH. DCU inhibited sEH-catalyzed tDPPO hydrolysis with a K_i of 34 nM. This is comparable to the K_i values for DCU with human (30 nM) and murine (26 nM) sEH (Morisseau, C. et al., Proc Natl Acad Sci USA 96, 8849-8854 (1999)).

Please replace paragraph [0076] with the following amended paragraph:

[0076] A study of the time course of the effect of a single dose of DCU (3 mg/kg) demonstrated that the antihypertensive effect in the SHR was acute (Figure 4C). Blood pressure was decreased 22 ± 4 mm Hg 6 hr after DCU treatment ($p < 0.01$) and returned to baseline levels by 24 hr after the dose. Importantly, there was no effect of DCU on blood pressure in the WKY (Figure 4D). This is consistent with the very low levels of sEH protein in the WKY kidney. Several additional structurally related inhibitors were also studied in the SHR. N-cyclohexyl-N'-dodecylurea is a sEH inhibitor with similar potency to DCU (IC_{50} with mouse sEH = 0.05 ± 0.01 compared to 0.09 ± 0.01 μ M for DCU; unpublished data, C. Morisseau and B. Hammock, 2000). A single dose of N-cyclohexyl-N'-dodecylurea significantly decreased systolic blood pressure 12 ± 2 mm Hg 6 hr after the dose, and similar to DCU, blood pressure returned to normal by 24 hours after the dose (Figure 5). The N-cyclohexyl-N'-ethylurea analog is a weak sEH inhibitor (IC_{50} with mouse sEH = 51.7 ± 0.7 μ M; unpublished data, C. Morisseau and B. Hammock, 2000) and had no effect on blood pressure in the SHR. Likewise, the selective mEH inhibitor dodecylamine also had no effect on blood pressure. Collectively, these data suggest that the effect of DCU and N-cyclohexyl-N'-dodecylurea on blood pressure is related to their ability to inhibit sEH and EET hydrolysis in vivo.